REMARKS

A check for the fee for filing a three month extension of time accompanies this response. The fee for filing the RCE can be charged to Deposit Account No. 02-1818. Any fees that may be due in connection with this application throughout its pendency may be charged to Deposit Account No. 02-1818. If a Petition for extension of time is needed, this paper is to be considered such Petition.

A supplemental Information Disclosure Statement is filed under separate cover.

Claims 1, 6-8, 21-23, 40, 46, 137, 307 and 341, 343, 345 and 347-361 are pending in the application. Claims 1, 6, 7, 21, 46, 307, 348 and 356 are amended and claims 5 and 279 are cancelled without prejudice, and claims 357-361 are added. Claim 1 is amended to include the limitations of claim 5, and also to render it clear that the polypeptide is not produced in any host that would recognize an E41Q mutation as a glycosylation site; claim 6, which previously depended on claim 1, is rewritten as an independent claim. Claims 357-361 capture subject matter that would be deleted by the amendment of claim 1 to include the limitations of claim 5 and the amendment of claim 6. No new matter is added. Non-elected and withdrawn subject matter is retained pending allowance of a linking claim.

CLAIM OBJECTIONS

Claim 23 is objected to as containing non-elected subject matter. Since the species election lead to the election of specie SEQ ID NO 87, the other SEQ ID NOs in the claim are not elected. In the interest of advancing prosecution of this application to allowance, claim 23 is cancelled.

THE REJECTION OF CLAIMS 1, 6, 17, 279, 341 AND 347 UNDER 35 USC 112, SECOND PARAGRAPH

Claims 1, 21, 40, 279, 341, 343, 347, 348 and 350 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention because it is alleged that one of skill in the art would not know what an interferon-alpha is. This rejection respectfully is traversed.

In the interest of advancing this application to allowance, amendment of claims renders this ground for rejection moot. Independent Claim 1 is amended by incorporation of the limitations of claim 5, which is not rejected on this basis; and independent claim 348 is amended to depend from claim 6, which is not rejected on this basis. All remaining claims depend from claim 1 or claim 6. Therefore, this ground for rejection is rendered moot.

Nevertheless, for reasons of record, Applicant respectfully continues to traverse this rejection. As discussed and demonstrated in the previous response; interferon-alpha cytokines are well-known to those of skill in the art. Applicant has provided a mutation that alters properties of such polypeptides, such that they exhibit increased bioavailability upon subcutaneous administration and are orally available. As shown in the application and in the previous response, the locus E41Q, is conserved. The specification clearly describes the members of the alpha interferon family. Further, such family is well known and is highly conserved. One of skill in the art would know when they had an interferon alpha, and would be able to determine the sequence to the position corresponding to position E41.

To evidence that one of skill in the art would knows what interferon alpha is, the results of a search of issued and published applications prior to the earliest priority date claimed in this application to was provided to demonstrate that one of skill in the art knows what an interferon alpha is. The previous response demonstrates that those of skill in the art as long as 20+ years ago were familiar with the members of this family as patents with claims reciting an "interferon-alpha" without reference to a sequence issued based on applications filed at that time. It is arbitrary to state that in 2001, when this application was filed, one of skill in the art, would suddenly be unable to assess that which one of skill in the art could assess in the 1980s.

2. Claim 279 is alleged to be indefinite because the meets and bound of "an interferon alpha structural homolog" cannot be assessed. As amended this language was cancelled from claim 279, rendering claim 279 duplicative with another claim, necessitating cancellation of claim 279. Nevertheless, Applicant continues to traverse this rejection, as the application is directed to, among other things, structural homologs of interferon-α2b. The application provides more than 1300 examples of structural homologs of interferon-α2b.

The Examiner urges:

> On pages 44-45 of the Remarks Applicant argues the specification is detailed enough for understanding of the recitation "structural homolog". The arguments were carefully considered but not found persuasive because as presented in the arguments (and absent a particular SEQ ID NO which may be identified structurally and functionally with a specific molecule) it may be construed that the mere three dimensional structure. of the compound claimed (structure which may be attained with a variety of amino acids residues) might not achieved the functionality of an interferon. Again, the lack of specificity of the mutations as defined in the context of a SEQ ID NO renders the claim indefinite.

As discussed in the previous response, the instant application is directed to two methods for producing polypeptides that have a predetermined property. In the first method,

a particular molecule, such as interferon-α2b, is employed and region(s) of interest determined *in silico*. The regions are then scanned, such as by Alanine scanning to identify residues that alter the property. Then, each such residue is replaced one-by-one with the remaining amino acids to identify any that confer the desired property.

In the second method, the 3-D method, polypeptides produce by the 2-D method are used to identify corresponding mutations in structural homologs by structural homology. About half the application is devoted to describing and teaching how to prepare structural homologs. The application defines structural homology and structural homologs, and then exemplifies them with the cytokines.

A substantial portion of the application is devoted to describing structural homologs of interferon-alpha and the application provides more than 1000 modified cytokines whose mutations are identified by structural homology. An **entire section** of the application, starting at page 91, is devoted to describing the interferon-alpha family member structural homologs and identifies the corresponding positions **in every known member** of the interferon alpha family. Hence, it is respectfully submitted, that anyone, whether of skill in the art or substantially lesser skill, would know what is meant by structural homolog in claim 279. It is arbitrary and capricious to say otherwise. The application clearly describes, defines what a structural homolog of interferon-α2b is, and identifies the interferon-α structural homologs (pages 74-76):

> **a. Structurally Homologous Interferon Mutants**
>
> Also provided herein are modified cytokines or cytokine structural homologues of IFNα-2b that are IFNα cytokines. These IFNα cytokines include, but are not limited to, IFNα-2a, IFNα-c, IFNα-2c, IFNα-d, IFNα-5, IFNα-6, IFNα-4, IFNα-4b, IFNα-I, IFNα-J, IFNα-H, IFNα-F, IFNα-8 and IFNα-consensus cytokine (see, SEQ ID No. 232). Accordingly, among the modified IFNα cytokines provided herein are those with one or more amino acid replacements at one or more target positions in either IFNα-2a, IFNα-c, IFNα-2c, IFNα-d, IFNα-5, IFNα-6, IFNα-4, IFNα-4b, IFNα-I, IFNα-J, IFNα-H, IFNα-F, IFNα-8, or IFNα-consensus cytokine corresponding to a structurally-related modified amino acid position within the 3-dimensional structure of the IFNα-2b modified polypeptides provided herein. The replacements lead to greater resistance to proteases, as assessed by incubation with a protease or a with a blood lysate or by incubation with serum, compared to the unmodified IFNα-2a.
>
> In particular embodiments, the modified IFNα cytokines are selected from among:
>
> the modified IFNα-2a that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 182, corresponding to amino acid positions: 41, 58, 78, 107, 117, 125, 133 and 159;
>
> the modified IFNα-c that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID

NO: 183, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

the modified IFNα-2c cytokine that is human and is selected from among cytokines comprising one or more single amino acid replacements in SEQ ID NO: 185, corresponding to amino acid positions: 41, 58, 78, 107, 117, 125, 133 and 159;

the IFNα-d modified polypeptide that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 186, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

the IFNα-5 modified polypeptide that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 187, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

the IFNα-6 modified polypeptide that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 188, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

the IFNα-4 modified polypeptide that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 189, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

the IFNα-4b modified polypeptide that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 190, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

the IFNα-I modified polypeptide that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 191, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

the IFNα-J modified polypeptide that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 192, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

the IFNα-H modified polypeptide that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 193, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

the IFNα-F modified polypeptide that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 194, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

the IFNα-8 modified polypeptide that is human and is selected from among polypeptides comprising one or more single amino acid replacements in SEQ ID NO: 195, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160; and

the IFNα-consensus modified polypeptide that is human and is selected from among polypeptides that contain one or more single amino acid replacements in SEQ ID NO: 232, corresponding to amino acid positions: 41, 58, 78, 107, 117, 125, 133 and 159.

Thus, the specification clearly and unequivocally identifies all structural homologs in the known family members, such that one of skill in the art would be able to assess whether a particular interferon-alpha included a E41Q. **Hence** when read in light of the specification, the meaning of "an interferon alpha structural homolog" is clear as is the identity of an interferon-alpha, which is used generically in a variety of issued patents, and is clearly defined in the instant application.

Nevertheless, in this application, in the interest of advancing claims to allowance, reference to structural homologs is deleted and all claims reference sequence identifiers, thereby obviating these rejections.

OBVIOUSNESS-TYPE DOUBLE PATENTING

Claims 1, 5-7, 16-19, 21-23, 40, 43, 44, 139, 141, 279, 307-308, 315, 316 and 332-347 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 68-70, 72-79, 81-82 84-86, 89-90 of copending Application No. 11/176,830. Although the conflicting claims are not identical, they are not patentably distinct from each other because they are both drawn to proteolytic resistant Interferon variants or compositions containing the same.

Because the instant application and the copending application are in prosecution, and the claims in one or both of the applications may be amended or one application abandoned, it is premature to file a terminal disclaimer. The copending application includes generic claims to compositions formulated for oral administration that contain cytokines modified in their primary sequence, whereby the cytokine is protease resistant. Dependent claims recite that the cytokine is an interferon alpha. The possibility for obviousness-type double patenting in the instant application and the copending application only exists with respect to these dependent claims in the copending application. If claims in the instant case, which are directed to interferon alpha cytokines with a modification at E41Q (or the corresponding locus), are allowed, claims to this family can be cancelled in the copending application voiding the need for terminal disclaimer in the instant application. Accordingly deferral of resolution of this issue respectfully is requested.

THE REJECTION OF CLAIMS 1, 6, 7, 23, 279, 307-308, 315, 316, 341, 342 AND 344-347 UNDER 35 U.S.C. §103(a)

Summary

As discussed in previous responses, the instant claims provide interferon-alpha cytokines that contain a mutation at E41Q. By virtue of this mutation in the primary

sequence, they exhibit properties neither taught nor suggested by any art of record. These results are demonstrated in the DECLARATION of Vega of record and discussed previously. In particular, the claimed modified interferon-alpha cytokines exhibit increased bioavailability upon subcutaneous administration, and, by virtue of this single mutation, they can be orally administered. None of the art of record, singly or in any combination, teaches or suggests or even hints at a desire to make an oral interferon-alpha; none teaches or suggests any way to do so, and none teaches or suggests that this can be achieved by a single amino acid change or even by rendering the polypeptide more resistant to proteases. This is the first example in the art that it is even possible to modify an interferon-alpha to render it orally available. This is achieved by replacing E at residue 41 with Q. All claims require this modification. The claim unequivocally states that it is the modification in the primary amino acid sequence that increases protease resistance. Claim 1 recites:

> . . . an amino acid replacement is E41Q, whereby the interferon alpha cytokine exhibits increased resistance to proteolysis compared to the unmodified interferon alpha cytokine that does not comprise the amino acid replacement . . .

To further advance prosecution, and to render this clear, the claim, as amended, recites that the E41Q position is not glycosylated. The polypeptide used in the experiments described in the DECLARATION was not glycosylated at E41Q; the results are achieved by virtue of the change in the primary sequence, not because the polypeptide is modified by glycosylation.

There is no art of record that teaches or suggests modification of the primary sequence to render a therapeutic polypeptide orally available, nor that increasing protease resistance can do so. There is no art of record that teaches or suggests modification of the primary sequence of any therapeutic polypeptide to increase protease resistance, and there is clearly and unequivocally no teaching or suggestion for replacing E41 with Q in an interferon alpha nor the results achieved thereby. Such change does not create a glycosylation site in any host of which the applicant is aware, and the **tested** polypeptide in the Declaration is **not** glycosylated at the E41Q locus. Thus, to conclude that one ordinary skill in th art somehow would have selected E41 to mutate and replace with a Q, and would have expected provide oral bioavailability because it allegedly introduces a site that some enzyme could glycosylate *in vitro*, does not teach or suggest the results shown in the DECLARATION that the polypeptide that does not contain any glycosylation at E41Q exhibits increased half-life and oral bioavailability. There is no basis in the art to conclude that any pending claim is *prima facie* obvious.

As discussed previously, while *prima facie* obviousness for the E41 to Q replacement is not established, Applicant provided the DECLARATION to advance prosecution and show that by virtue of an E to Q replacement at position 41, interferon-alpha cytokines possess properties that are not taught or suggested by the cited art. As described in the DECLARATION, the results show that the exemplary mutant E41Q not only exhibits increased protease resistance to a cocktail of proteases, but also to blood lysate, serum and to chymotrypsin. Thus, the amino acid replacement confers increased protease resistance of the interferon-alpha cytokine as a whole, which increased resistance is not specific to a particular protease nor to a particular putative protease cleavage site.

In the DECLARATION, data also are provided that demonstrate that the polypeptides exhibit improved pharmacokinetics upon *subcutaneous and oral administration* compared to polypeptides not containing the amino acid replacement(s). For example, the DECLARATION provides data demonstrating that a mutant IFN-alpha containing only a single amino acid mutation (E41Q), when administered subcutaneously or orally, retains anti-viral activity in the serum for a longer time period than the native polypeptide. ***With respect to per-oral administration, the native polypeptide exhibits NO detectable activity when administered orally; whereas, the IFN-α with a single amino acid change, can be successfully administered orally***. There is nothing in the art that suggests that such achievement is possible, nor suggests how to modify an IFN-α to achieve such results.

In addition, the results show that SuperLEADs, containing two or more amino acid changes described in the above-captioned application, also exhibit similar increases in half-life. The Declaration also shows that the polypeptide retains activity with the change in primary sequence

The combinations of references cited in the rejections, discussed below, do not in any manner, combine to result in the instantly claimed modified interferon-alpha cytokines nor suggest the results achieved thereby. None, singly or in any combination, teaches or suggests modification of E41Q; none teaches or suggests modification of the interferon-alpha to render it orally available; none teaches or suggests modification of the primary sequence to increase protease resistance; none teaches or suggests the results achieved thereby. Not only does combining the references rely on the teachings of the application for their combination; even when combined they do not teach or suggest the instantly claimed modified interferon alpha polypeptides. There is no showing of *prima facie* obviousness.

It is an anathema to the constitutional mandate (Article 1 section 8) upon which the patent laws are based to maintain these rejections. The inventor is entitled to a period of exclusivity for developing such a valuable therapeutic. As stated in the previous response, the undersigned respectfully states that the Office in its diligence to avoid issuing unwarranted patents, should not be over zealous in rejecting all claims. To do so is stifling to this industry and destructive to early stage and emerging companies with truly innovative products. Orally available interferons are of significant value to the public; rejecting claims on unsustainable bases and prolonging prosecution can destroy early stage companies and can deprive the public of the benefits of the developments.

None of the cited art, singly or in any combination, teaches or suggests that modification of this locus is sufficient by itself to substantially increase in resistance to proteases, retain biological activity and improve pharmacokinetics when administered subcutaneously and orally. The Declaration demonstrates these results using an unglycosylated interferon-alpha the contains only this single change (further amino acid changes can be introduced, but the single change at E41Q is sufficient by itself). Further none of the cited art, singly or in combination with any or all of the cited art, teaches or suggests that, by virtue of this change interferon-alpha cytokines can be administered orally and exhibit activity; whereas interferon-alpha cytokines that do not contain this modification do not exhibit any activity upon oral administration. The DECLARATION shows that a single change from E to Q at position 41 in an interferon alpha results in the improve pharmacokinetics upon subcutaneous administration and also permits the polypeptide to be formulated, for example, as tablet or capsule, for oral administration. No art of record even suggests that rendering a polypeptide protease resistant by virtue of changes in its primary sequence permits oral delivery, nor does the art teach or suggest the improved properties observed upon subcutaneous administration. The cited art does not teach, suggest or even hint at such extraordinary results. If the art suggests anything, it suggests that IFN-alphas contain a plurality of sites that are cleaved by proteases; the art does not teach or suggest that changing E41 to Q will render the polypeptide orally available. Hence, as discussed below in addressing the rejections under 35 U.S.C. §103(a), the Examiner has failed to set forth a *prima facie* case of obviousness. The combination of teachings of the references does not lead to the instantly claimed polypeptides. Notwithstanding that, the DECLARATION certainly provides data sufficient to rebut any *prima facie* case of obviousness of the instant claims.

Claims 1, 5-7, 21-22, 40, 279, 307, 341, 343, 345 and 347-356

Claims 1, 5-7, 21-22, 40, 279, 307, 341, 343, 345 and 347-356 are rejected under 35 U.S.C. 103(a) as being unpatentable over Heinrichs *et al.* view of Jensen *et al.* (WO/01/36001, 05/25/2001-cited in the previous Office action) in further in view of Walter MR (Seminars in Oncology, 24, S9-52-S9-62, 1997), Bernkop-Schnurch (J. Controlled release, 52,116,1998), Blank *et al.* (Eur. J. Biochem., 265, 11-19, 1999-cited in the previous Office action), Sheppard P. (U.S. Pat. 6,153,420-cited in the previous Office action) Yan *et al.* (Biochemistry, 23, 3759-3765, 1984) and Black *et al.* (J. Biol. Chem. , 264, 5323-5326, 1989). Each of the references and the comments of the Examiner are addressed in turn below. The Examiner concludes:

> It would have been obvious for a person of ordinary skill in the art at the time that the invention was made to combine the teachings of Heinrichs *et al.* with the teachings Jensen *et al.*, Walter MR, Bernkop-Schnürch, Blank *et al.*, Sheppard *et al.*, Yan *et al.* and Black *et al.* to obtain IFN-α compounds and compositions containing surface exposed residues that are protected and thus conferring resistance to proteolysis with a reasonable expectation of success. This is because the exposed site are known and also proteases that are encountered at the site where the composition would be administered is uncovered by Sheppard *et al.* or at sites where the compounds would be present (like in the blood) is disclosed by Black *et al.* The motivation to do so would have been offered by the advantages of the compositions taught by Heinrichs *et al.* and Jensen *et al* with respect to the proteolytic resistance of the modified mutants. Also the teaching of Jensen *et al.*, read in the light of Walter and Yan *et al.* would have made it obvious for a person of ordinary skill in the art to try a limited number of surface exposed positions in the IFN-α to be mutated to obtain better products. The E41 is just one of the limited residues that could be tried because, on one hand, by mutating it first eliminates a potential site for glutamyl endopeptidases (like the enzyme taught by Sheppard) which would degrade the IFN-α 2 and thus make it unusable for therapy. Such as enzyme, found in small intestine and colon, would severely impede the absorption of IFN-α 2 and increase the dosage necessary for therapy and thus could reach its toxicity limit. A person of ordinary skill in the art would have had a finite number of amino acids to choose from changing the E41. An additional motivation to choose the glutamine (Q) as a mutation for E41 is the finding of Yan *et al.* that glutamine can be glycosylated and the glycosylated product might have an easier task of being internalized in the cell. Once the IFN-α has passed into the blood stream it might have encountered a protease as described by Black *et al.* and thus protection against it would become obvious by employing the teaching of Walter and mutate the D94 site. The choice for Glycine would assure a minimal surface exposure given the small size of this amino acid. Thus, a comprehensive reading of the references would necessarily lead a person of ordinary skill in the art to make the substitution E41Q and D94G since a skilled artisan has good reason to pursue known options in her or his technical grasp.

This rejection respectfully is traversed.

In the discussion below, each of the cited references is discussed, including the Examiner's comments regarding each reference. Following the discussion of each reference

the combined teachings and the deficiencies thereof are discussed. Applicant is not attacking each reference separately; but to assess the deficiencies of the combination of teachings, each reference is considered.

To summarize the discussion below, none of the cited references, singly or in combination teaches or suggests:

1) that a single mutation in primary sequence of interferon-alpha is sufficient by itself to result in a polypeptide that exhibits increased resistance to any protease;
2) that such single mutation could result in an orally available interferon;
3) which residue to modify to achieve this result; and/or
4) the results achieved by replacing E41 with Q in an interferon-α.

Furthermore, to render it clear that is the change in the primary sequence that confers the properties on the polypeptide, not glycosylation, the claims are amended to clearly recite that the E41Q locus is not glycosylated.

The Examiner's appears to indicate that if someone decided to modify interferon-alpha, eventually they would modify E41 and replace it with a Q. The Examiner appears to be stating that if someone decided to modify all residues in an interferon-alpha, eventually they would modify E41 and replace it with a Q. There is no evidence that one of skill in the art would, in light of the art, expect that this residue is sufficient by itself to render the polypeptide resistant to a cocktail of proteases and orally available. To say that the art, which provides disparate teachings regarding proteases, and modifications of proteins, and the structure of interferon-alpha polypeptides, does not lead inexorably to the conclusion that that E41 is a key residue to change. The art would suggest that one must change a plurality of residues, including introduction of glycoslyation consensus sequences and glycosylation of the polypeptide, at a plurality if sites.

The standard for obviousness is not whether someone, if they decided to do what applicant did, would eventually invent the same thing; nothing would be patentable under that standard. Furthermore, even if that were the standard, in this instance, none of the cited art even mentions anything about modifying an interferon-alpha to render it orally available without glycosylation by virtue of the modification, nor even that there is a problem that interferon-alpha is not orally available. Hence, the ordinarily skilled artisan would not even have embarked on the experiments that the Examiner contends would lead to the instantly claimed polypeptides.

In a post-KSR decision, PharmaStem Therapeutics. Inc. v. ViaCell. Inc., 491 F.3d 1342 (Fed. Cir. 2007), the Federal Circuit stated that:

> an invention would **not** be invalid for obviousness if the inventor would have been motivated to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. Likewise, an invention would not be deemed obvious if all that was suggested was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

Furthermore, KSR has not overruled existing case. See In re Papesch, (315 F.2d 381, 137 USPQ 43 (CCPA 1963)), In re Dillon, 919 F.2d 688, 16 USPQ2d 1897 (Fed. Cir. 1991), and In re Deuel (51 F.3d 1552, 1558-59, 34 USPQ2d 1210, 1215 (Fed. Cir. 1995)). "In cases involving new compounds, it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound." *Takeda v. Alphapharm*, 492 F.3d 1350 (Fed. Cir. 2007).

In this instance, none of the cited art suggests that it is desirable to modify the primary sequence of an interferon-alpha and that upon doing so it will be orally available. None teaches or suggests that changing only a single amino acid is sufficient to achieve this. None of the cited art teaches or suggests modification of E41 at all; none suggest modification to Q and none suggest the results achieved thereby. Furthermore, none of the cited art even provides a suggestion to or desire to produce an orally available interferon-alpha. Hence there is no teaching that would have led one ordinary skill in the art to vary any parameters to produce a modified interferon-alpha that includes only a single amino acid change sufficient to render it orally available and to increase its bioavailability upon subcutaneous injection, while retaining biological activity. As discussed below, there is nothing in the art that points to replacement of E41 with any amino acid, none that (reasonably) would suggest Q, and none the results achieved thereby.

For completeness, the results are demonstrated in the Declaration of Vega, which has been discussed previously, and summarized again.

The Declaration of Dr. Vega demonstrates results not taught or suggested by the combination of teachings of the cited references.

As discussed previously and below with respect to the new ground of rejection, notwithstanding the fact that the combination of teachings of the references fails to teach or suggest modifying a polypeptide to increase protease resistance, the combination of teachings

of the reference does not teach or suggest the results achieved thereby. These results include: (1) modification of E41Q alone, without post-translational modification, confers resistance to a cocktail of proteases; (2) modification can be achieved without substantially altering a desired biological activity; (3) polypeptides, which are not orally available become so upon modification of as little as amino acid (E41Q) and that such oral availability does not require glycosylation ; and (4) the polypeptides so-modified also exhibit improved pharmacokinetics upon subcutaneous administration.

The application, and Declaration, describe that a cytokine, including an interferon alpha cytokine, exhibiting increased resistance to proteolysis, when administered subcutaneously or orally, exhibit increased stability and half-life and thus exhibit improved pharmokinetics compared to a cytokine not containing the modification. None of the cited references, singly or in any combination teaches or suggests such results. The Declaration shows that while wild-type interferon-alpha has no activity when administered orally, interferon-alpha with as little as just the E41Q modification, is orally available. This is quite amazing.

As discussed below, the references would suggest that a plurality of changes are required to confer resistance to proteases and that, for oral availability, the polypeptide requires modification that adds glycosylation sites and formulation with protease inhibitors.

Each of the reference is first discussed, and then the combination of teachings is discussed. Applicant is not attacking each reference piecemeal, but is first describing each reference and its deficiencies, which can then be summed up to assess whether the combination teaches or suggests the claimed polypeptides and results achieved thereby.

Teachings of each of the cited references and deficiencies of each, including rebuttal to the Examiner's comments (the combined teaches are discussed below).

Heinrichs *et al.*,

The Examiner states that Heinrichs *et al.* teaches methods for identifying IFN-α homologs that have altered properties or activities such as enhanced antiviral, antiproliferative, growth inhibitory, cytostatic, cytotoxic activity or reduced immunogenicity. The Examiner states that Heinrichs *et al.* also teaches that the resulting polypeptides can be further modified, for example, to increase serum half-life, reduce antigenicity, or increase polypeptide stability by glycosylation, sulfation, PEGylation and phosphorylation. The Examiner states that the mutants:

> . . . possess clinical utility in that they are designed towards optimization for use as pharmaceuticals and to overcome dose-limiting toxicity, receptor cross-reactivity, and short serum half-lives significantly reduce the clinical utility of many of these cytokines The existence of abundant naturally occurring sequence diversity within the interferon-alphas (and hence a large sequence space of recombinants) along with the intricacy of interferon-alpha/receptor interactions and variety of therapeutic and prophylactic activities creates an opportunity for the construction of superior interferon homologues (p. 2-3).

The Examiner continues and states that Heinrichs *et al.* teaches a directed evolution method by which one *could* identify interferon mutants that have enhanced antiviral, antiproliferative, growth inhibitory, cytostatic, cytotoxic activity or reduced immunogenicity, or having other property such as low immunogenicity, increased half-life, improved solubility or oral availability. As such, the Examiner concludes that "Heinrichs present evidence of the ***feasibility*** of obtaining IFN compounds with better characteristics, the motivation to do it and some means of accomplishing the goals, for instance for greater oral availability. Also as mentioned, in the previous action, Heinrichs *et al.* do not specifically teach the mutation E41Q or D94G."

As discussed previously and below, Applicant emphasizes that neither Heinrichs *et al.*, nor any cited reference teaches or suggest **modifying the primary sequence** of an interferon-alpha for oral administration nor for increasing half-life or serum stability. Heinrichs *et al.* teaches gene shuffling methods for identification of interferon-alphas that have altered antiviral, antiproliferative, growth inhibitory, cytostatic, cytotoxic activity or reduced immunogenicity. Heinrichs *et al.* **does not mention** oral availability nor modifying the primary sequence to increase protease resistance. All of the mutants identified by Heinrichs *et al.* contain a plurality of modifications in the sequence needed to change the antiproliferative activity or similar activity of the interferon-alpha. There is no hint that a single amino acid change could suffice.

Heinrichs *et al.* does not teach or suggest mutation at E41Q, as required by all pending claims. In addition, in the extensive body of art in this area, which is of record in this application, no one has ever so-modified an interferon-alpha. The methods of Heinrichs *et al.* necessarily result in a polypeptide with a plurality of changes; whereas, the instant application and Declaration of record show that as little as only the E41Q mutation is required to confer the demonstrated properties on an interferon-alpha.

Heinrichs *et al.* also teaches that the resulting polypeptides can be further modified to increase serum half-life, reduce antigenicity, or increase polypeptide stability and teaches that such modifications include glycosylation, sulfation, PEGylation, phosphorylation. These are

not modifications of the primary sequence per se, but require modification of the polypeptide by addition of a non-polypeptide moiety.

Heinrichs *et al.* 1) does **not teach** that increased serum half-life can be achieved by altering the primary sequence, but teaches that such is achieved by polypeptide modifications, such as glycosylation; 2) does not teach that oral bioavailability can be achieved by modification of the primary sequence of the polypeptide nor suggest how to achieved such; and 3) does not hint at or even suggest using gene shuffling methods to do this or that any gene shuffling method results in a polypeptide with **as few as only a single amino** acid change in its primary sequence (as shown in the Declaration) that can be administered orally and that has increased bioavailability upon subcutaneous administration.

Hence, Heinrichs *et al.*, which merely teaches applying gene shuffling methods to interferon-alpha to alter antiviral, antiproliferative, growth inhibitory, cytostatic or cytotoxic activity or reduced immunogenicity, is of little relevance to the instant claims, which are directed to particular interferon-alpha mutants that have a change at E41 that results in the properties discussed above. Heinrichs *et al.* is of marginal relevance to the instant claims.

Jensen *et al.*

The Examiner states that Jensen *et al.* teaches interferon-gamma compounds have extended half-life in vivo and improved stability towards proteolysis (p. 4, lines 1-6) by virtue of insertion adding glycosylation or PEGylation or other such pendant moiety, and that such compounds can be formulated in a pharmaceutical composition in a variety of forms, including formulated for oral administration are specifically taught (p. 42, line 33 to p. 43, line 29). The Examiner states that Jensen *et al.* also teaches the introduction of an E38N mutation, which results in creation of a glycosylation site consensus sequence because of the presence of a Serine at position 40, and that E38N is alleged by be exposed at least 50% to the surface of the polypeptide (p. 16, line 32 to p.17, line 2). The Examiner states that "the most important teaching of Jensen is that the E38 of the IFN gamma is at a surface exposed polypeptide site, an exposed site that is a prerequisite for a proteolytic attack."

First, at page 4, lines 1-6 Jensen *et al.* states that the conjugates (IFN-gamma modified to have linked non-polypeptide moieties) can have increased *in vivo* half life and optionally improved stability towards proteolysis. It does not state at this page, nor anywhere, that modification of any residue, with or without glycosylation, can render the IFN-gamma orally available, and it certainly is silent regarding modification of the primary sequence without concomitant glycosylation.

As discussed in the previous response, Jensen *et al.*, does not teach or suggest anything regarding modification of an interferon-alpha nor teach nor suggest modifying any polypeptide to render it orally available. Jensen teaches introducing mutations in a surface exposed site to create glycosylation sites glycosylation or PEGylation or other such moiety to increase half-life of interferon-gamma, but does not teach or suggest a change in sequence that itself increases resistance to proteases and also permits oral administration of the polypeptide.

Jensen *et al.* does not teach or suggest that only a single amino acid change in any polypeptide, and certainly not an interferon-alpha, can increase, by virtue of the change in the amino acid sequence, not by virtue of glycosylation, protease resistance or increase bioavailability upon subcutaneous administration or permit oral administration.

The instant claims specifically recite that it is the change in the primary amino acid sequence that confers the increased protease resistance as well as the oral availability, not glycosylation of any residue. To further emphasize the claim specifically recites that the polypeptide is not glycosylated at E41Q. Thus, Jensen *et al.* is of no relevance to the instant claims.

Walter

The Examiner states that "Walter teaches that the residues E41, D94 and R23 are exposed residues in the molecule of Interferon alpha (IFN α) (figure 4) and thus exposed to a proteolytic attack." It respectfully is submitted that Walter does not provide such teaching; Figure 4 indicates that quite a few residues have 50% or more solvent accessibility, not just these three residues.

Walter provides a three dimensional model of interferon-alpha 2B crystal structure and states that its structure is a paradigm for the structure of the entire family. Nowhere in Walter does it teach or suggest modification of E41, nor does it state that "E41, D94 and R23 are exposed residues" or that they are exposed to a proteolytic attack. Figure 4 indicates that that **these residues are among at least 29 residues** are solvent accessible, but does not call

out E41, D94 or R23 residues nor implicate them in susceptibility to proteolytic cleavage. Figure 4 indicates that there are other solvent exposed Glu residues, including, for example, E79, E113 and E132. E42 is characterized as having 21%-50% solvent accessibility and Blank *et al.* shows that it actually is cleaved by Glu-C.

Figure 4 also indicates that E41 is a residue that buries surface area in the dimer interface in the crystals. Walter clearly does not mention modification of any of these residues. In discussing the crystal structure at page 53, Walter states that the N-terminal five residues, the C-terminal six amino acids, and residues 103-111(the CD loop) and also residues 45-50 in the AB3 loop are solvent exposed

Besides the sequence set forth in Figure 4, the only statement in Walter regarding E41, occur on page 53, column 2, which states that E41 and E42 form the zinc binding site in the dimers that form in $ZnAc_2$ solutions, pH 5.6. No other mention is made of residue E41, but such role in the dimer structure, would not suggest that E41 is a candidate for modification.

Bernkop- Schnürch

The Examiner states that Bernkop-Schnürch teaches that for sufficient bioavailability of therapeutic agents after oral dosing, several barriers encountered with the gastrointestinal (GI) tract have to be overcome. One of these barriers is caused by proteolytic enzymes, leading to a severe presystemic degradation in the GI tract (abstract).

Bernkop-Schnürch is directed to the **use of inhibitory agents** in formulations to overcome the barriers to bioavailability of polypeptide therapeutics after oral dosing. Bernkop-Schnürch states that **one** of these barriers is caused by proteolytic enzymes in the GI tract, and states that co-administration of inhibitory agents is a solution to the problem. Bernkop-Schnürch teaches that the inhibitory agents that are to be co-administered depend upon the structure of the polypeptide (*i.e.*, the presence of particular cleavage sites for the variety of proteases to be encountered) and the inhibitors available for each that can cleave the particular polypeptide. Hence Bernkop-Schnürch teaches that polypeptides contain a variety of cleavage sites for the variety of proteases encountered in the GI tract, that one can identify the sites that are present, and include the appropriate inhibitor in the composition when administering the compositions. Bernkop-Schnürch is silent regarding modification of polypeptide sequence to eliminate protease cleavage sites, but certainly implies that a polypeptide will not be susceptible to a single protease but to a variety that cleave at different sites.

Hence there can be no expectation from Bernkop-Schnürch that a single amino acid change in a polypeptide would render a polypeptide resistant to all proteases in the GI tract, further evidencing the unexpected nature of the results achieved by the instantly claimed polypeptides.

Bernkop-Schnürch provides no teachings or suggestions for oral administration of any interferon.

Blank *et al.*

Blank *et al.* teaches possible cleavage sites for a Glu-C protease in the IFN α-2b molecule (Fig. 5), which Blank *et al.* employs for identifying epitopes in IFN α-2b. The Examiner says that it includes the E41 residue as putative site [although the paper, as discussed previously does not show it as an actual protease cleavage site], for Glu-C protease (as indicated by the boxed residues).

As discussed in the previous response, each E residue in the interferon polypeptide is identified as a "possible" cleavage cite for Glu-C, but Blank *et al.* in fact does not demonstrate that E41 is a cleavage site for this protease. Blank *et al.* is directed to the identification of epitopes in IFN-α2b that confer binding to four different anti-IFNα2b monoclonal antibodies, including identification of the sequence recognized by the antibodies by comparing the immunoreactivity of various proteolytically digested fragments; and thus uses the locus of the proteolytic cleavage sites as markers for identification of the epitopes. The E41Q site is not cleaved in the described experiments.

To the extent Blank *et al.* teaches anything related to proteases, Blank *et al.* teaches the ***possible*** cleavage sites in IFN-α2b following digestion with Arg-C (C-terminal end or arginine residues), Glu-C endoprotease (C-terminal end or glutamate residues) and prolyl endopeptidases (C-terminal end or proline residues.) Specifically, Blank *et al.* teaches that *any* glutamic acid (E) is a potential cleavage site for Glu-C, *any* arginine (R) is a potential cleavage site for Arg-C and *any* proline (P) is a potential cleavage site for prolyl endopeptidases in the amino acid sequence of IFNα2b (see Figure 5). Since position 41 in the amino acid sequence of IFN-α is Glu (E), it was identified as a ***possible*** cleavage site by Glu-C endoprotease. Following digestion with each of the proteases, however, Blank *et al.* identified **actual cleavage site**s based on N-terminal sequencing of corresponding digested immunoreactive fragments. ***Glu41 is not among the sites shown to be cleaved.*** Rather, **Glu42** is identified as a cleavage site and it is shown in Walter to be partially solvent accessible.

Blank *et al.* shows that there are 14 Glu resides in the interferon-alpha. If all 13 E internal residues in the interferon-alpha are cleaved by the Glu-C endoprotease, there is no reason to possibly conclude that modification of one them would eliminate cleavage of any other sites. Even applying the Examiner's logic regarding solvent exposed residues, E41 is not the only solvent exposed E residue in the interferon-alpha (see figure 4 of Walter; i.e. E79, E113, E132); leads to the conclusion that at least 4 residues would have to be modified to eliminate cleavage by the Glu-C endoprotease, not just one residue. Coupling this finding with Bernkop-Schnürch, which teaches that the GI tract contains a panoply of proteases that cleave throughout polypeptides, there would be no expectation from Blank *et al.* nor any cited reference that elimination of a single site would somehow render that polypeptide orally available or even protease resistant. The instant claims only require modification of E41Q; this change, as shown in the Declaration and described in the application is sufficient by itself to render the polypeptide resistant to a cocktail of proteases, as well as orally available and having increased bioavailability upon subcutaneous administration.

Sheppard

Sheppard teaches a serine protease that appears to be homologous to glutamyl endopeptidases (Glu-C proteases) found in tissues exposed to the external environment, like small intestine and colon (col. 5, line 21 to col. 6, line 31), which, the Examiner, not the Shepard, states would be the site of degradation of an orally administered composition. Sheppard provides no such teaching. Sheppard *et al.* is directed to a serine protease, Zsig13, which was identified by querying an EST database. Sequence analysis revealed homology to serine proteases, and sequence alignment with other proteases confirmed such homology. Probing of tissue samples indicates that this protease may expressed in the trachea, bladder, small intestine, colon and prostate. Sheppard *et al.* states that this data "suggest that it could be a digestive or anti-bacterial protease." Since it is a protease identified from an EST by homology, and has not been isolated nor shown to even occur in the digestive tract, it is a leap to suggest that one of ordinary skill in the art would select render a therapeutic polypeptide resistance to such protease for any reason. Further, Sheppard *et al.* does not teach or suggest that modifying a polypeptide to be resistant to this **putative** digestive or bacterial enzyme nor that rendering a polypeptide resistant thereto would render a polypeptide, previously inactive upon oral administration, active, nor that it would improve the pharmacokinetics upon subcutaneous administration. Sheppard *et al.* states that potential utility of Zsig13 is to degrade unwanted polypeptides in industrial processes.

Sheppard does not teach or suggest modification of the primary sequence of any polypeptide that would render it protease resistant, nor does Sheppard teach or suggest any modification of the primary sequence that permits oral administration of an alpha interferon. Sheppard does not teach or suggest that Glu-C proteases are the only proteases that would cleave an interferon-alpha nor that the only site cleaved by Glu-C would be E41. As discussed above, Blank teaches that there are 14 potential cleavage sites for Glu-C in interferon alpha, and 4 of them (E41, E79, E113, E132) are solvent exposed. Shepard does not teach or suggest that elimination of a single Glu-C site would render any polypeptide resistant to the protease, which can cleave a plurality of sites, nor is there any suggestion that eliminating one or all protease cleavage sites would render any polypeptide orally available.

In addition, as discussed above, Bernkop-Schnürch teaches that the GI tract contains a panoply of proteases that cleave throughout polypeptides and suggests that multiple proteases can cleave a polypeptide. Bernkop-Schnürch teaches adding inhibitors of the proteases that cleave to the composition. Hence identification of one protease that may be present in the digestive tract provides no insights regarding residues that will be cleaved, nor any expectation regarding the minimum number of changes required to render a polypeptide protease resistant.

Yan *et al.*

Yan *et al.* is alleged to teach introduction of glycosyl units at glutamines by transglutaminases and thus facilitate the internalization of the glycopolypeptides (Abstract and p. 3759, right col., lines 1-5 of the article).

Yan *et al.* does not teach that the studied transglutaminases should be used as way of glycosylating polypeptides nor that any polypeptide should be mutated to include a Q and then glycosylated with this enzyme *in vitro*. Yan *et al.* is a study investigating requisites for the activity of the transglutaminases for this purpose.

Yan *et al.*, which is a 1984 publication, investigated the use of a transglutaminase for preparing neoglycopolypeptides. Using β-casein as a substrate for study, Yan *et al.* found that not all glutamine residues were glycosylated, and examined the sequences around the acceptor sites in β-casein. Yan *et al.* concludes that hydroxy amino acids (T, S, Y) appear to be adjacent to the Glu in 5 of the 7 acceptor sites and all receptor sites are located in either the random coil or β-bend of the then-predicted structure of β-casein. No definitive conclusions regarding the sequence requisites for recognition of a particular Glu residue as an acceptor site, other than that there are additional requisites, such as an adjacent hydroxy

amino acid, and that not all Glu residues serve as acceptor sites. Hence, as taught by Yan *et al.* changing a single residue to Glu will not necessarily create a site recognized by a transglutaminase for its use to glycosylate polypeptides *in vitro*.

E41 is **not** adjacent to a hydroxy amino acid. Comparison with Figure 5, which sets forth the sequence of β-casein shows that the sequence EQ in the polypeptide (as is present in the modified interferon-alpha molecules) is not glycosylated by this enzyme. In view of Yan *et al.*, one cannot find motivation to modify to introduce a Q into a polypeptide to create a glycosylation site, since Yan *et al.*, teaches that more is required. Hence in view of Yan *et al.*, it is unlikely that E41Q would serve an acceptor, since their data evidences the more than just a Q is needed and that EQ is not an acceptor site. The skilled artisan would not mutate E41 to Q in an effort to produce a glycosylation site for any enzyme.

Furthermore, a search of the literature to determine whether anyone ever has actually glycosylated polypeptides *in vitro* using a transglutaminase, did not yield any hits, nor could the undersigned find follow-up references to Yan *et al.* (published in 1984) that provide insights into the requisites for use of a transglutaminase to glycosylate polypeptides. Thus, it does not appear that this is a viable method that one of ordinary skill in the art would employ to produce glycosylated polypeptides. Clearly in view of Yan *et al.*, one of ordinary skill in the art, on the even remote chance that such person would glycosylate polypeptides using a transglutaminase, would not merely introduce a Gln residue and expect it to be glycosylated by the transglutaminase without further changes in the polypeptide sequence to create an appropriate concensus sequence. No art of which Applicant is aware teaches introducing a glycosylation site by merely replacing an amino acid with Q. Those of skill in the art desirous of introducing a glycosylation site, would introduce an O- or N- glycosylation site by creating a consensus sequence therefor.

Further, while claim specifically recites that this it change in the residue to Q that confers the protease resistance on the entire polypeptide. In addition, render this clear the claim also recites that that E41Q is not glycosylated. As discussed above, the data demonstrating that an interferon-alpha polypeptide containing an E41Q mutation possesses unexpected properties was generated with a polypeptide that was not glycosylated. Hence, the Declaration clearly shows that the modified interferon-alpha **does not** derive its protease resistance nor oral availability by virtue of glycosylation.

If the Examiner is suggesting that upon ingesting an interferon-alpha with an E41Q mutation it will be glycosylated *in vivo,* neither Yan *et al.* nor any art of record suggests a

mechanism whereby an ingested polypeptide is glycosylated. There is no such teaching in Yan *et al.* nor is a possible mechanism or chance that such happens *in vivo* provided in Yan *et al.*, or any cited reference.

Black *et al.*,

The Examiner states that Black *et al.*

> "teach a pre-aspartate specific protease from the human leukocytes (abstract). The protease was identified in the conditioned medium from human peripheral blood mononuclear cells and is unique in its requirement of an aspartate as a recognition site since the enzyme is not active even if such mutations of the aspartate is made with very similar amino acids like glutamate or asparagine. (Discussion section)."

Black *et al.* employed the precursors of interferon-1β as a substrate to detect proteolytic activities in peripheral blood monocytes to identify any that may be involved in processing interleukin-1β from its inactive precursor form to active form. The activity that effects cleavage of interferon-1β to render it active was purified. Black *et al.* concludes that the enzyme that effects this cleavage is specific for pre-aspartate cleavages. Black *et al.* states that the enzyme does not cleave at E or N residues. E41 is **not adjacent to a D residue.** Certainly, if the enzyme identified in Black *et al.* cleaves an interferon-alpha, it does not do so at E41. Further, Black *et al.*, teaches that the observed highly specific cleavage specificity "suggests a role in processing rather than in general polypeptide degradation." Thus, there is nothing in Black *et al.* to suggest that this enzyme plays a role in proteolytic degradation. Thus, the relevance of this reference is unclear, since the observed activity is a processing enzyme.

The combination of teachings of the cited references does not result in the instantly claimed interferon-alpha polypeptides that contain an E41Q mutation, nor do they teach, suggest or even hint at the results achieved by virtue of this mutation.

As discussed above, Heinrichs *et al.* provides gene shuffling methods for identification of interferon-alphas that have altered antiviral, antiproliferative, growth inhibitory, cytostatic, cytotoxic activity or reduced immunogenicity. Heinrichs *et al.* does not mention oral availability nor modifying the primary sequence to increase protease resistance. All of the mutants identified by Heinrichs *et al.* contain a plurality of modifications in the sequence needed to change the antiproliferative activity or similar activity of the interferon-alpha. There is no hint that a single amino acid change could suffice.

Jensen *et al.*, which allegedly teaches replacement of a solvent exposed residues in interferon-gamma, teaches that introduction of a glycosylation site and production of a IFN-gamma modified to have linked non-polypeptide moieties can have increased in vivo half life

and optionally improved stability towards proteolysis, nor oral availability. Jensen *et al.* does not teach or suggest modifying any residue other than modifying residues to create a glycosylation site or other site to link non-polypeptide moieties. Walter identifies at least 29 residues that are solvent accessible. Even if Walter somehow teaches the skilled artisan that certain residues are exposed to the surface of the molecule, it does not suggest which among them to change, nor to which amino acid to replace it nor that replacing only one such residue permits oral administration. Combination with Jensen, would suggest modification of residues to create a consensus sequence for glycosylation; none of the residues indicated in Figure 4 (those with 3 underneath them) are amenable to modification by a single change to create a glycosylation site, including for glycosylation using a transglutaminase as allegedly taught by Yan, which teaches that more than just a Q is required for a site to be glycosylated by a transglutaminase.. Further, there is no teaching in Jensen for a change without concomitant glycosylation.

Blank *et al.*, which identifies epitopes in interferon-alpha, shows that there are 14 Glu resides in the interferon-alpha. If all 13 E residues in the interferon-alpha are cleaved by the Glu-C endoprotease, there is no reason to possibly conclude that modification of just one them would eliminate cleavage at any other protease sites. The Examiner in citing Blank *et al.* as as well as Black *et al.* identifies 12 other Glu-C cleavage sites as well as aspartate residues for cleavage by the activity described by Black *et al.* Even applying the Examiner's logic regarding solvent exposed residues, E41 is not the only solvent exposed E residue in the interferon-alpha (see figure 4 of Walter, which shows that at least three additional E residues E79, E113, E132 are solvent accessible); leading to the conclusion that at least 4 residues would have to be modified to eliminate cleavage by the Glu-C endoprotease, not just one residue. Coupling this finding with Bernkop-Schnürch, which teaches that the GI tract contains a panoply of proteases that cleave throughout polypeptides, and Black *et al.*, which according the Examiner, teaches a protease that cleaves before aspartate resides, there would be no expectation to elimination of a single site would someone render that polypeptide orally available or even protease resistant.

Bernkop-Schnürch teaches that polypeptides contain a variety of cleavage sites for the variety of proteases encountered in the GI tract, that one can identify the sites that are present, and include the appropriate inhibitor in the composition when administering the compositions. Bernkop-Schnürch is silent regarding modification of polypeptide sequence to

eliminate protease cleavage sites, but certainly implies that a polypeptide will not be susceptible to a single protease but to a variety that cleave at different sites.

Yan *et al.* teaches that a transglutaminase can glycosylate polypeptides *in vitro* at particular Q residues, but does not teach that it glycosylates any and all Q residues. As discussed above, Yan *et al.*, suggests that there are additional requisites for a receptor site for a transglutaminase. Further, there is no evidence of record that anyone has ever mutated a polypeptide to introduce a Q residue for glycosylation with a transglutaminase. Glycosylation sites are introduced by modification of polypeptides to contain consensus sequences for N- or O- glycosylation. It is a stretch to conclude that one of ordinary skill in the art would read Yan *et al.* and decide to change E41 or any residue to a Q in order to glycosylate a polypeptide or that such is feasible or practical. Further, the claims specifically recite that the E41Q mutation results in the increased stability, oral availability and other such properties, not glycosylation. Hence, if one of ordinary skill in the art decided to mutate an E to a Q to somehow create a glycosylation site, the result that the resulting polypeptide does not require glycosylation to exhibit various properties is unexpected.

As noted above, Black *et al.* teaches that proteolytic cleavage in peripheral blood monocytes is required for activation of interferon-1β and an enzyme that effects this cleavage is specific for pre-aspartate cleavages. The activity identified by Black *et al.* is stated by Black *et al.* to be a processing enzyme, not a degradative enzyme. Black *et al.* purifies this activity by measuring the activity of interferon-1β as an assay and shows that it cleaves at residues adjacent to aspartates. Thus, Black *et al.* does not appear to be relevant to the instantly claims, which all require a modification at E41Q, which by itself is sufficient to render the polypeptide sufficiently protease resistant to increase bioavailability upon subcutaneous injection and to render it suitable for oral administration.

As discussed, Blank *et al.* states that there are 14 potential Glu-C cleavage sites and demonstrates that Glu42 is a protease cleavage site, but does not demonstrate that Glu41 is cleaved. Further, since it demonstrates that there are 14 potential sites, there is no suggestion in Blank *et al.* nor any cited reference that elimination of one site renders the entire polypeptide resistant. In addition, neither Blank *et al.* nor any reference of record teaches or suggests that Glu-C is the only protease present in the gut or serum that would cleave an interferon-alpha polypeptide. In fact, Black *et al.*,, cited by the Examiner, discusses a protease that cleave at pre-aspartic acids, and Shepard indicates that a plurality of proteases cleave polypeptides upon oral administration and suggests. Jensen *et al.*, which is directed to

interferon-gamma conjugates and teaches introducing N- or O- glycosylation sites by modifying in the interferon-gamma to include a consensus sequence therefor, teaches that the resulting polypeptide should be glycosylated to increase stability. As shown in the instant Declaration, glycosylation is not required to achieve the protease resistance of the polypeptide that comprises the E41Q modification.

Sheppard *et al.* teaches a putative protease identified from an EST library that may be expressed in the digestive tract. Sheppard *et al.* does not teach or suggest that rendering a polypeptide resistant to this proteases or any glutamyl endopeptidase would permit oral administration of such polypeptide, nor does it teach or suggest eliminating only one potential protease cleavage site renders the entire polypeptide resistant to cleavage by same or other proteases at non-modified sites. Sheppard *et al.*, or any cited reference, does not teach or suggest that these are the only proteases present in the gut. Further, the instantly modified interferon-alpha polypeptides exhibit improved pharmacokinetics upon subcutaneous administration. Modification of E41 does not result in a loss of relevant biological activity.

Thus, combination of teachings of these references does not lead one to change E41 in an interferon-alpha (there are a plurality of exposed residues) nor to change it to a Q (does not result in a glycosylation site; the claim polypeptide specifically exhibits increased protease resistance by virtue of the change in the primary sequence, not glycosylation), nor the results achieved thereby, including oral availability. The Examiner is reminded that unexpected properties must always be considered in setting forth a *prima facie* case of obviousness. In this instance, the art cited by and as interpreted by the Examiner teaches that there are a plurality of protease cleavage sites in interferon-alpha polypeptides and that protection can be achieved by glycosylation. As discussed repeatedly throughout this response, none of the art teaches or suggests that it only is necessary to change a singe amino acid, E41, in an interferon-alpha to render the it resistant to a plurality of proteases that cleave at a plurality of sites. None of the art teaches or suggests modification of a single site to prepare orally administratable interferon-alpha. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

The Examiner concludes, however, that:

> [i]t would have been obvious for a person of ordinary skill in the art at the time that the invention was made to combine the teachings of Heinrichs *et al.* with the teachings Jensen *et al.*, Walter MR, Bernkop-Schnürch, Blank *et al.*, Sheppard *et al.*, Yan *et al.* and Black *et al.* to obtain IFN-α compounds and compositions containing surface exposed residues that are protected and thus conferring resistance to proteolysis with a reasonable expectation of success. This is because the exposed site

> are known and also proteases that are encountered at the site where the composition would be administered is uncovered by Sheppard *et al.* or at sites where the compounds would be present (like in the blood) is disclosed by Black *et al.* The motivation to do so would have been offered by the advantages of the compositions taught by Heinrichs *et al.* and Jensen *et al* with respect to the proteolytic resistance of the modified mutants. Also the teaching of Jensen *et al.*, read in the light of Walter and Yan *et al.* would have made it obvious for a person of ordinary skill in the art to try a limited number of surface exposed positions in the IFN-α to be mutated to obtain better products.

It respectfully is submitted that this conclusion is flawed in numerous respects for the reasons addressed above. To summarize and address the Examiner points:

a. The Examiner states:

> [i]t would have been obvious for a person of ordinary skill in the art at the time that the invention was made to combine the teachings of Heinrichs *et al.* with the teachings Jensen *et al.*, Walter MR, Bernkop-Schnürch, Blank *et al.*, Sheppard *et al.*, Yan *et al.* and Black *et al.* to obtain IFN-α compounds and compositions containing surface exposed residues that are protected and thus conferring resistance to proteolysis with a reasonable expectation of success.

As discussed above, Heinrichs *et al.* does not teach or suggest producing proteolytically resistant polypeptides by virtue of a change in the primary sequence, particularly a change the can be effected with only a single amino acid change. Heinrichs *et al.* discusses increasing stability by glycosylating polypeptides. Similarly Jensen *et al.* teaches modifying interferon-gamma at surface exposed residues to be glycosylated. The instant claims specifically require that the amino acid change (E41Q) confers the increased resistance to proteolysis, not glycosylation. The Declaration demonstrates that this change, without glycosylation confers the observed properties. Walter , Bernkop-Schnürch, Blank *et al.*, Sheppard *et al* teaches that there are 29 exposed residues, 14 potential cleavage sites by Glu-C proteases including at least 4 (E41, E78, E113 and E132) that are surface exposed, and that a panoply of proteases can cleave polypeptides in the GI tract. Walter indicates that E41 is important for dimer formation and Zn binding, thus, suggesting modification of this residue could alter biological activity. This combination does not lead one of ordinary skill in the art to modify E41 and to expect that the resulting polypeptide, which has a plurality of exposed residues will be resistant to a cocktail of proteases and be orally available.. At most it suggests that protease resistance will require changes at a plurality of sites and the glycosylation sites should be introduced. Even if one reads Yan *et al.* as teaching an *in vitro* glycosylation method; its teachings suggest that a plurality of residues should be changed and that changing any residue to a Q does not result in a site for transglutamination. In its example, β-casein, not all "Q" residues were glycosylated; the sequence "QE" was **not**

glycosylated by the transglutaminase. Thus, in view of Yan *et al.*, even if for some reason one of ordinary skill in the art decided to introduce glycosylation sites for glycosylation using a transglutaminase, one of ordinary skill in the art would not have any expectation that E41QE42, as is present in the modified interferon-alphas would be an acceptor site for the transglutamination, since such site was not effective as described in Yan *et al.*.

Also, as discussed, the instant claims specifically recite that the change in amino acid sequence of E41Q confers the resistance, not glycosylation. None of the references suggest that changing only a single site could render the polypeptide protease resistant. None of the references teaches or suggests rendering a polypeptide orally available by changing its primary sequence. The only mention of oral administration is Bernkop-Schnürch, which teaches formulation of polypeptides with a plurality of protease inhibitors to try to achieve oral availability. Bernkop-Schnürch does not teach or suggest that modification of the primary sequence of a polypeptide could render it orally available. Further, Bernkop-Schnürch teaches that one has to identify all potential protease cleavage sites and include inhibitors for a plurality of proteases. Hence, there would be no expectation that changing only a single amino acid would be sufficient to render a polypeptide protease resistant.

b. The Examiner continues:

> [i] This is because the exposed site are known and also proteases that are encountered at the site where the composition would be administered is uncovered by Sheppard *et al.* or at sites where the compounds would be present (like in the blood) is disclosed by Black *et al.* The motivation to do so would have been offered by the advantages of the compositions taught by Heinrichs *et al.* and Jensen *et al* with respect to the proteolytic resistance of the modified mutants.

As discussed above, there Walter teaches that at least 29 residues are solvent accessible, but does not call out any particular residues nor implicate them in susceptibility to proteolytic cleavage. There are 29 exposed sites, and no suggestion that changing only one site would, without glycosylation, render a polypeptide protease resistant. Blank *et al.* teaches that there are 14 E residues in interferon-alpha, certainly shows that residues other than E41 are cleaved are cleaved by Glu-C, does not teach or suggest that elimination of E41 will confer resistance to Glu-C and does not teach or suggest that Glu-C is the only protease encountered in the GI tract. Heinrichs *et al.* and Jensen *et al.*, specifically teach modification of polypeptides by glycosylation to achieve increased stability. The instant claims clearly state that increased resistance is not achieved by glycosylation nor is that change one that introduces a glycosylation site. Thus, the cited references do not lead to E41 as **the** site to change, nor to a change to a Q residue. In fact, if one of ordinary skill in the art were seeking

to introduce a glycosylation site, it is highly unlikely that such person would not introduce it as everyone else does by modifying the sequence to contain a consensus sequence for O- or N- glycosylation.

c. The Examiner concludes that one of ordinary skill in the art would have picked out the surface-exposed residues and would have changed them and somehow would have happened upon E41Q mutation.

> The E41 is just one of the limited residues that could be tried because, on one hand, by mutating it first eliminates a potential site for glutamyl endopeptidases (like the enzyme taught by Sheppard) which would degrade the IFN-α2 and thus make it unusable for therapy. Such as enzyme, found in small intestine and colon, would severely impede the absorption of IFN-α2 and increase the dosage necessary for therapy and thus could reach its toxicity limit. A person of ordinary skill in the art would have had a finite number of amino acids to choose from changing the E41. An additional motivation to choose the glutamine (Q) as a mutation for E41 is the finding of Yan *et al.* that glutamine can be glycosylated and the glycosylated product might have an easier task of being internalized in the cell. Once the IFN-α has passed into the blood stream it might have encountered a protease as described by Black *et al.* and thus protection against it would become obvious by employing the teaching of Walter and mutate the D94 site. The choice for Glycine would assure a minimal surface exposure given the small size of this amino acid. Thus, a comprehensive reading of the references would necessarily lead a person of ordinary skill in the art to make the substitution E41Q and D94G since a skilled artisan has good reason to pursue known options in her or his technical grasp

It respectfully is submitted that even post-KSR, this in **not** the standard for obviousness. As quoted above, in a post-KSR decision, PharmaStem Therapeutics. Inc. v. ViaCell. Inc., 491 F.3d 1342 (Fed. Cir. 2007), the Federal Circuit stated that:

> an invention would not be invalid for obviousness if the inventor would have been motivated to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. Likewise, an invention would not be deemed obvious if all that was suggested was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

In this instance, the prior art does not even provide a teaching for producing an orally available form of interferon-alpha. The only mention of oral availability is Shepard, which teaches formulating polypeptides with a plurality of inhibitors of proteases that can cleave the polypeptide. Glu-C is far from the only protease that can cleave IFN-alpha, and there is no teaching or suggestion for modifying its primary sequence to eliminate such cleavage.

Further, there is no teaching in any reference that elimination of one potential Glu-C cleavage site would render the polypeptide resistant to all proteases in the GI tract. As discussed above, Yan *et al.* does not teach that any Q residue can be glycosylated by the transglutaminase, and shows, in β-casein that QE is **not** glycosylated.

Regarding Black it teaches a processing enzyme that activates interferon-1β. There is no relevance in the teachings of Black to the instant claims, which all require modification of E41. If it is cited regarding modification of an aspartate residue, there is no suggestion in Black that this activity accounts for proteolytic degradation of any polypeptide in the blood.

Furthermore, to combine these references to result in the instant claims relies on the improper use of hindsight

No reading of this art would lead one of ordinary skill to make an interferon-α with an E41Q modification. As discussed above, these disparate references do not teach or suggest modification of the primary sequence of a polypeptide to render the polypeptide protease resistant. The references teach modification of the polypeptide, which the instant claims clearly exclude at E41Q. Further, the references suggest that the polypeptide is susceptible to protease cleavage at a plurality of sites, not a single site and not a single protease. As shown in the application and DECLARATION interferon alpha with the E41Q modification and no glycosylation is resistant to a cocktail of proteases as wells as various serum and other proteases. The only reference that discusses oral formulations, teaches formulation of polypeptides with protease inhibitors; there is no suggestion for modification of the primary sequence of any polypeptide to render it protease resistant.

"To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

Thus, the only way the Examiner can combine the teachings of the cited references is to rely on the instant application as guide for selecting the references and modifying their teachings. The cited references must provide some teaching or suggestion to do that which applicant has done. This is absent in the instant case.

Finally, **unexpected properties**, detailed throughout this response, **must** be considered when setting forth a case of *prima facie* obviousness. In this instance, the application and Declaration as outlined several times above, clearly and unequivocally show

results not taught or suggested by the cited rat. As discussed above, the tested modified interferon-alpha contained only a modification at E41 and was not glycosylated. The art, that allegedly hones in on E41, cited by the Examiner also teaches that there are 29 surface exposed residues, that there are panoply of proteases that can cleave polypeptides *in vivo*, that interferon-alpha contains at least 4 exposed Glu resides, that E41 may be important in dimer formation and is a buried residue in the dimers, that there are 14 E residues in interferon-alpha that can be cleaved by only one protease from among the panoply of proteases to which the polypeptide will be exposed. There would no expectation that modification of E41 is sufficient alone to render the polypeptide resistant to a cocktail of proteases, to render it orally available, to increase bioavailability upon oral administration, and to retain biological activity. Thus, modification of E41, **whether alone or with other** residues, confers properties on an interferon-alpha that are not taught or suggested by the cited art.

Therefore, for any and all of these reasons, the Examiner has failed to set forth a *prima facie* case of obviousness.

Rebuttal to further remarks of the Examiner

1. The Examiner states that:

> On page 33 of the Remarks Applicant argues that, in the reference, that in Jensen *et al.* it is the conjugates, not the polypeptides, which exhibit improved stability towards proteolysis, which is correct. Applicant argues that Jensen *et al.* also does not teach or suggest that its conjugates, by virtue of increased protease resistance can be administered orally and rendered orally available. This is factually incorrect since compositions for oral administration are specifically taught (p. 42, line 33 to p. 43, line 29).

The compositions of Jensen *et al.* are not compositions of an interferon-gamma polypeptide, but contain conjugates of interferon-gamma. There is no suggestion in Jensen *et al.* for formulating unconjugated polypeptides for oral administration. The instant claims recite that the E41Q mutation confers the change in properties of the polypeptide, not glycosylation. Further, the instant Declaration shows that the modified interferon-alpha that is not glycosylated exhibits resistance to a cocktail of proteases. Such results are clearly unexpected in view of Jensen *et al.*, which teaches that glycosylation is a required.

2. The Examiner states that:

> Further, Applicant concludes that "Jensen *et al.* fails to teach virtually any of the elements of the instantly claimed compositions." The arguments were carefully considered but not found persuasive because the most important notion underscored by Jensen *et al.* and explained in the previous Office action is that is that the E38 of the IFN-gamma is at an polypeptide surface exposed site and it is desirable to protect the surface exposed sites that might be sensitive to proteolysis (or glycosylation for example in Jensen *et al.*'s case).

As discussed throughout this response, the Declaration shows that the modified interferon-alpha is not "protected" as taught by Jensen *et al.* It is not glycosylated at that site nor any site. Thus, the teachings of Jensen are inapposite.

3. The Examiner states

> On page 34 of the Remarks Applicant argues that Sheppard *et al.* provides no[t] (sic) teachings or suggestions that are of any relevance to the instant claims.
>
> The arguments were carefully considered but not found persuasive because as iterated in the previous Office action, Sheppard teaches a novel serine protease homologous to glutamyl endopeptidases (Glu-C proteases) which are found in tissues exposed to the external environment, like small intestine and colon (col. 5, line 21 to col. 6, line 31), which would be the site of degradation of an orally administered composition. The person of ordinary skill in the art does not have to have the same motivation as the Sheppard *et al.* to use the Glu-C protease as exactly taught by them. The rational trend of thought would be that, if this protease is encountered in the digestive tract, it would represent a set back for therapy procedures that are based on orally administered compounds which have exposed E residues. Thus, while administered to the gastro intestinal tract, the compound would be exposed to the attack of the Glu-C protease at a site taught by Blank *et al.*

As discussed above and previously, Sheppard *et al.* merely identifies a putative protease that it says may occur in the digestive tract. There is no teaching in Sheppard *et al.* or any reference of record that suggests that this putative protease cleaves an interferon-alpha at any E residue, nor that elimination of one potential cleavage site for this putative protease will render the entire polypeptide resistant to a cocktail of proteases and will permit oral administration of the polypeptide. As the art cited by the Examiner teaches, interferon-alpha contains at least three exposed E residues (E41, E113 and E132), with a number of others partially exposed, including E42, contains 14 E residues at which Blank *et al.*, teaches Glu-C can cleave, contains 29 surface exposed residues, which include among them sites for attack by other proteases.

4. The Examiner states:

> On page 32 of the Remarks Applicant argues that Blank *et al.* does not teach a Glu41 cleavage site in IFN α.
>
> The arguments were carefully considered but not found persuasive because Blank *et al.* teach possible cleavage sites for the IFN a-2b molecule (Fig. 5), which include the E41 residue, which, contrary to Applicants assertion in the remarks, is a cleavable site for Glu-C protease, as indicated by the boxed residues.

Blank *et al.* teaches that there are 14 residues at which Glu-C could cleave, but only shows cleavage at a subset of these residues, including E42.

5. The Examiner states:

> In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized

that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See In re McLaughlin, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

The above discussions show that the cited references do not lead to modification of E41 nor to modification of E41 to E41Q, and significantly, do not show the results achieved by such modification.

Claim 23

Claim 23 is rejected under 35 U.S.C. 103(a) as being unpatentable over Heinrichs *et al.*. (WO 01/25438, 04/12/2001-cited by the Applicant) view of Jensen *et al.*, (WO/01/36001, 05/25/2001) in further in view of Walter MR (Seminars in Oncology, 24, S9-52-S9-62, 1997, Bernkop-Schnürch (J. Controlled release, 52,1-16,1998), Blank *et al.* (Eur. J. Biochem., 265, 11-19, 1999), Sheppard P. (U.S. Pat. 6,153,420) Yan *et al.* (Biochemistry, 23, 3759-3765, 1984) and Pang DZD (U.S. Pat. No. 6,319,691).

Claim 23 is cancelled herein to advance prosecution of the subject matter directed to E41Q. It respectfully is submitted that this ground for rejection is rendered moot by cancellation of claim 23 herein.

* * *

In view of the above, examination of the application on the merits and allowance are respectfully requested.

Respectfully submitted,

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